

# Technical Explanation

## US Application 10/583,706

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# Claim 1 of the present application

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## (TP-FP primer set)

A primer set comprising at least two primers that allows a target nucleic acid sequence to be amplified,

wherein a **first primer** included in the primer set contains, in its 3' end portion, a sequence (Ac') that hybridizes to a sequence (A) located in the 3' end portion of the target nucleic acid sequence, and also contains, on the 5' side of the sequence (Ac'), a sequence (B') that hybridizes to a complementary sequence (Bc) to a sequence (B) that is present on the 5' side with respect to the sequence (A) in the target nucleic acid sequence, and

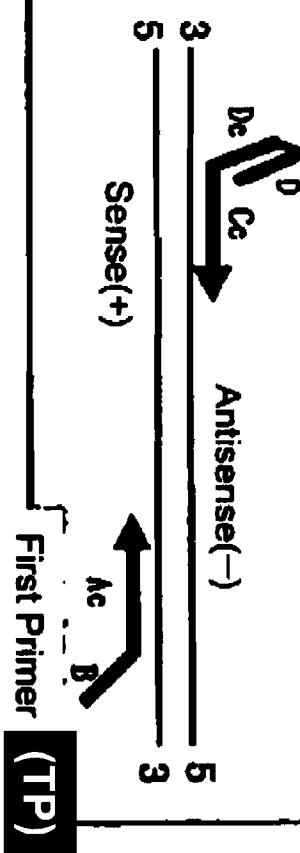
a **second primer** included in the primer set contains, in its 3' end portion, a sequence (Cc') that hybridizes to a sequence (C) located in the 3' end portion of a complementary sequence to the target nucleic acid sequence, and also contains, on the 5' side of the sequence (Cc'), a folded sequence (D-Dc') that contains, on the same strand, two nucleic acid sequences that hybridize to each other.

\*The first primer is **TP**, the second primer is **FP**.

**TP; Turn-back Primer**  
**FP; Folded Primer**

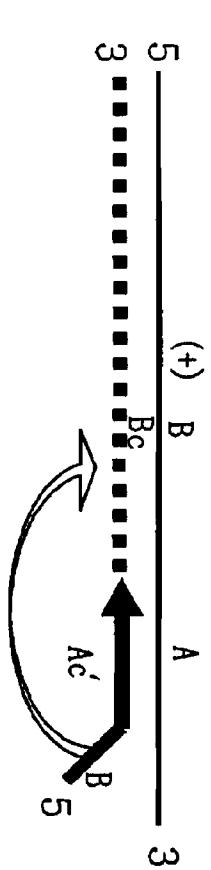
# Technical explanation of the TP and FP

## Second Primer (FP) Primer Set



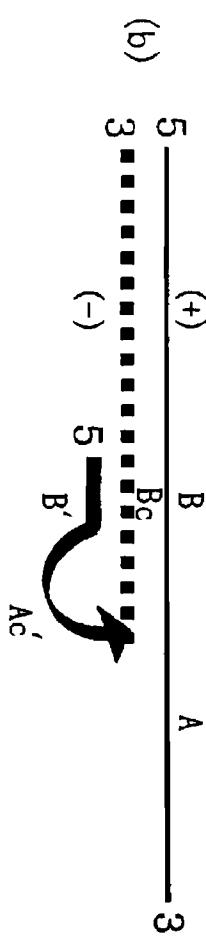
## TP has the function as follows;

- (1) TP has the turn back portion (B) in the 5' side sequence.
- (2) The turn back portion (B) can hybridize to the portion (Bc) of the elongation strand from TP.



**FP has the function as follows;**

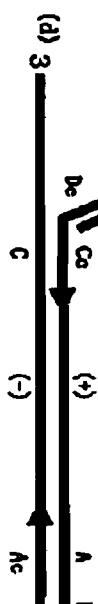
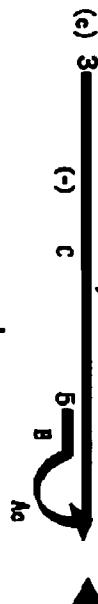
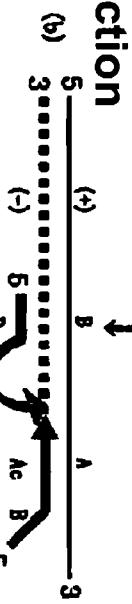
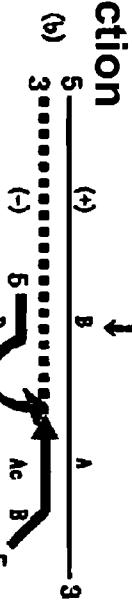
- (1) FP has the folded sequence (D-Dc') in the 5' side sequence.
- (2) The folded sequence (D-Dc') has two nucleic acid sequences that hybridize to each other.
- (3) The folded sequence (D-Dc') **DO NOT** hybridize to the elongation strand from FP.



## Mechanism of the amplification reaction of the TP-FP(1) (FIG.3 of the present invention)



Turn back  
reaction



Turn back  
reaction

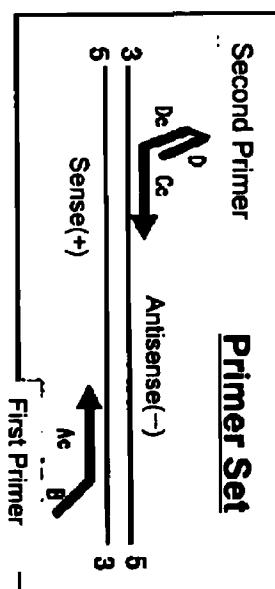


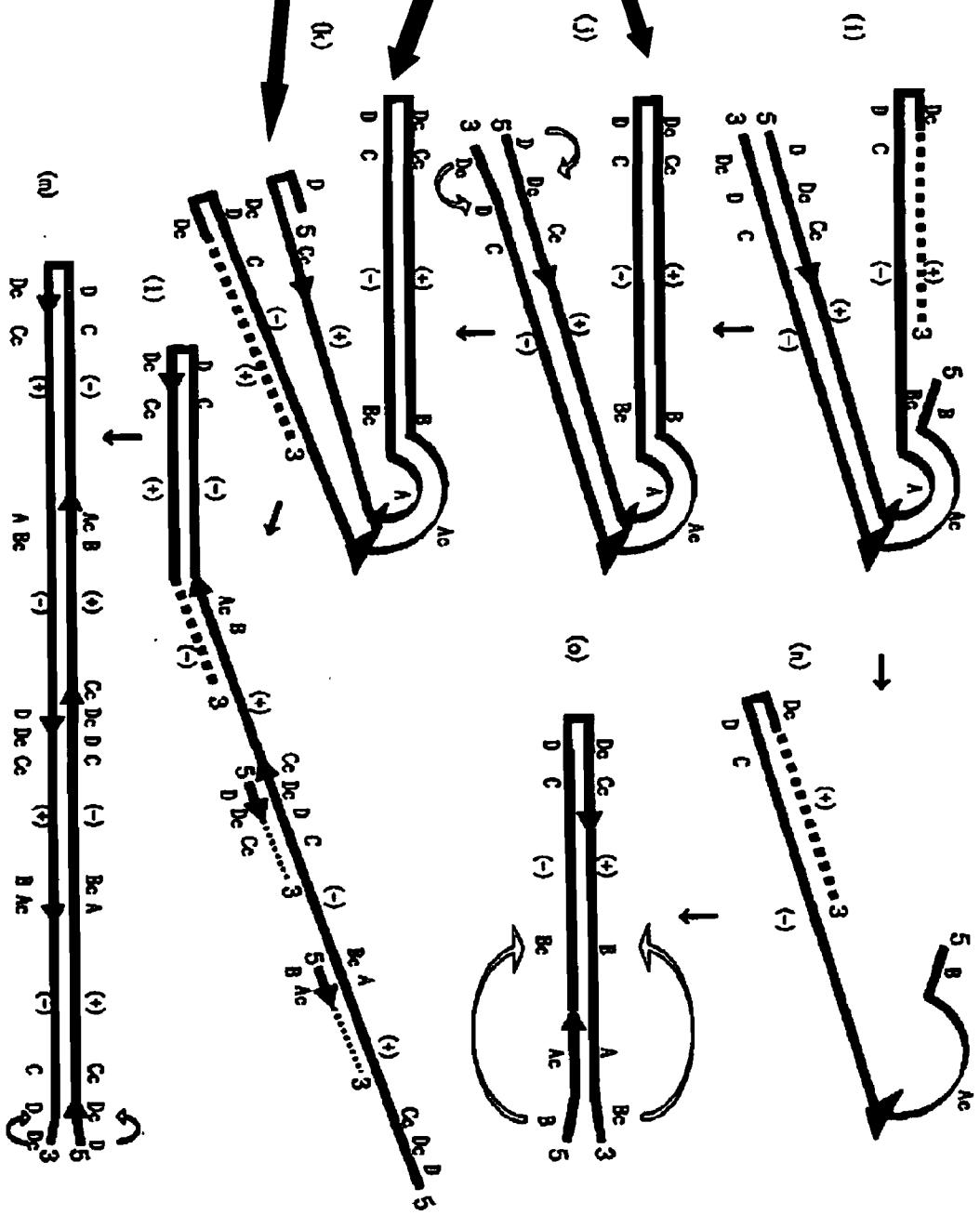
FIG. 3a

New TP  
hybridizes  
to the loop

that  
formed by  
elongation  
of the  
former TP.

## Mechanism of the amplification reaction of the TP-FP(2) (FIG.3 of the present invention)

TP DOES  
NOT  
hybridize  
to the  
folded  
portion  
formed by  
elongation  
of the  
former FP.



## **The present invention has four advantages.**

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### **(1) Isothermal amplification**

- The amplification occurs without thermal denaturation.

### **(2) Specific amplification**

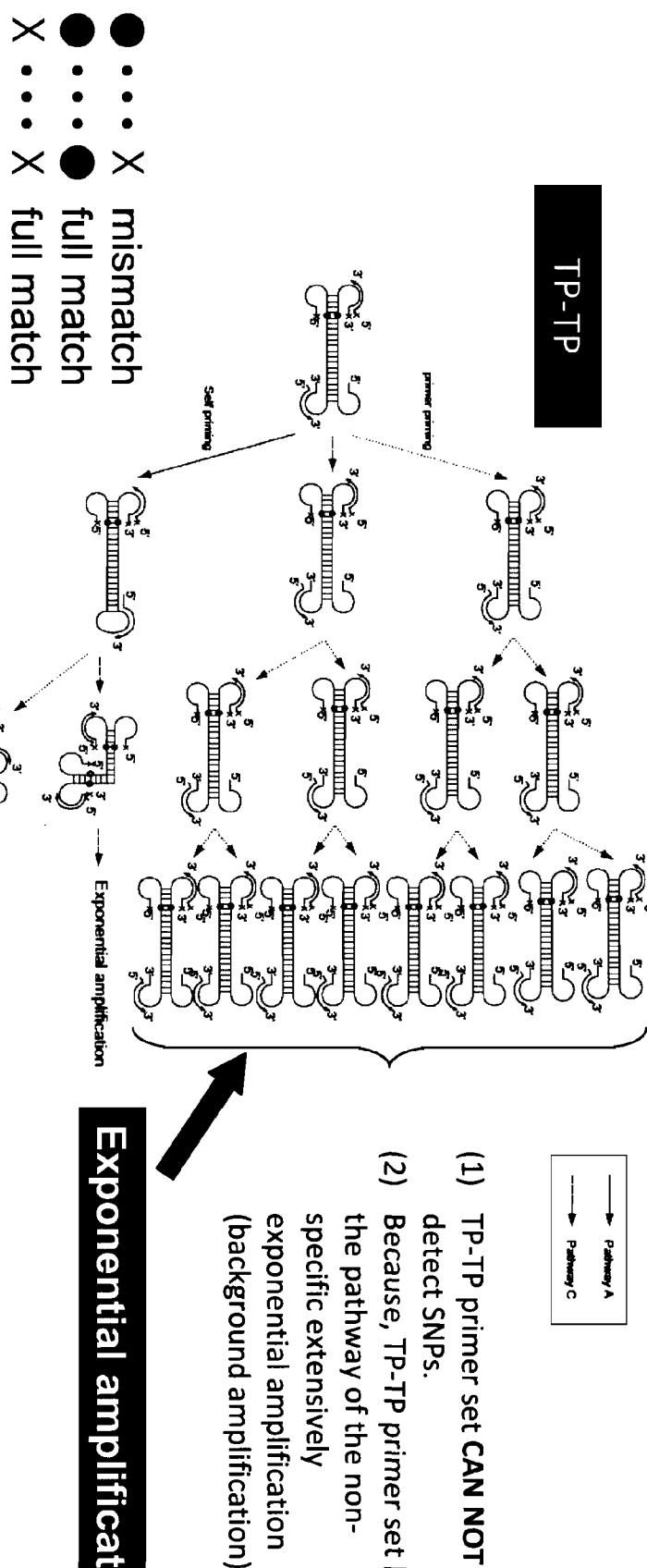
- The present invention can detect SNPs without non-specific amplification.

### **(3) Short time amplification**

### **(4) Easy primer design**

# Mechanism of specific amplification

The non-specific amplification **DOES NOT** occur in the present invention (TP-FP).

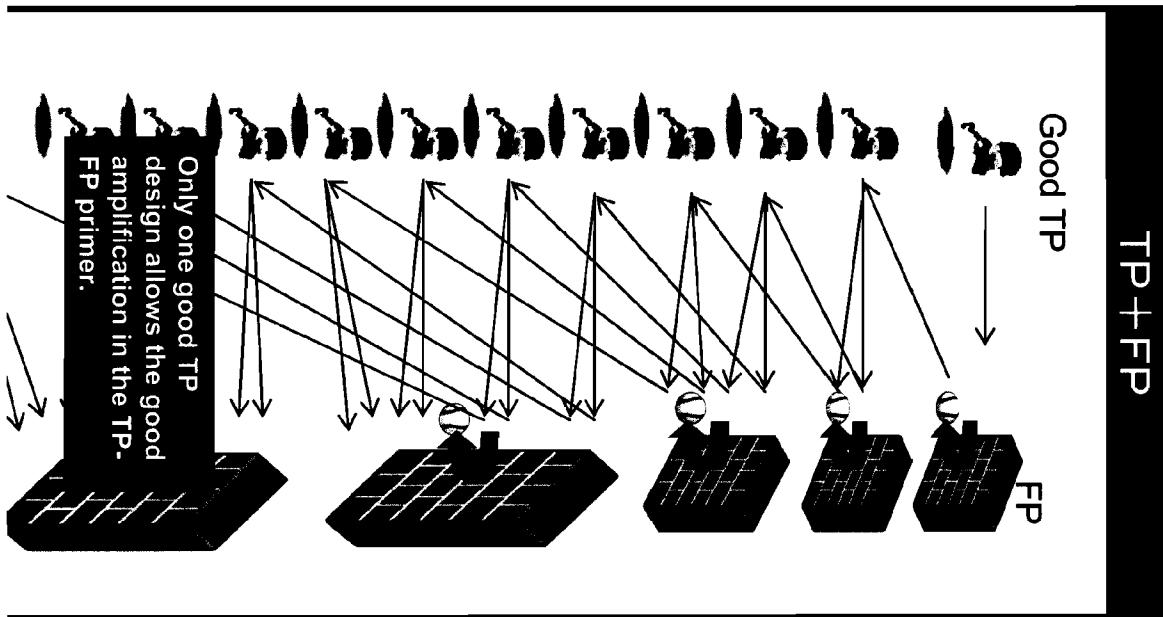
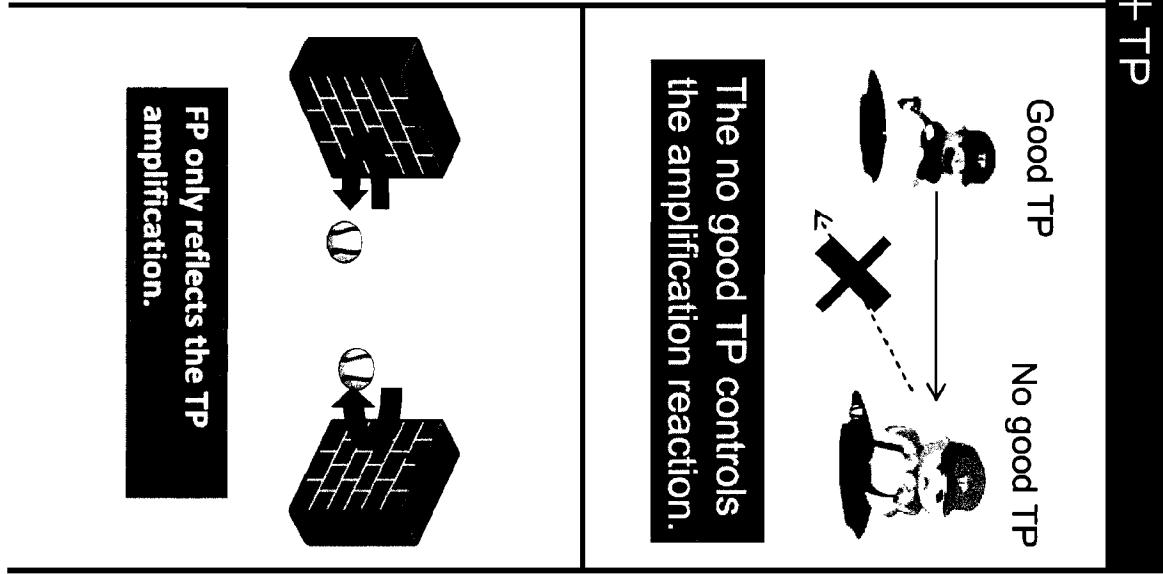
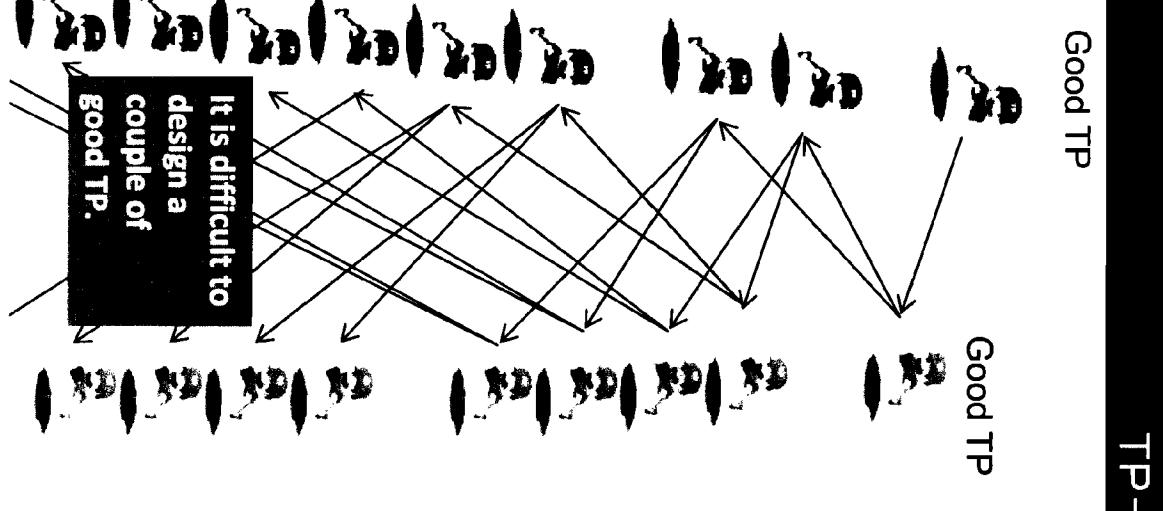


- (1) TP-FP primer set **CAN** detect SNPs.
- (2) Because, the non-specific exponential amplification of the pathway of TP-FP primer set is **very gentle**.

# Short time amplification and easy primer design(1)

- (1) TP
  - (i) TP can amplify exponentially.
  - (ii) TP has a strong engine of amplification.
  - (iii) TP has two area depending on template sequence.
- (2) FP
  - (i) FP can not amplify exponentially, but amplify linearly.
  - (ii) FP is like a mirror which reflect TP amplification.
  - (iii) FP needs only one area depending on template sequence.
- (3) TP-TP primer set
  - (i) TP-TP primer set needs four areas depending on the template sequence.
  - (ii) TP-TP Primer set needs a design of a couple of good TP because the reaction is totally controlled by no good TP.
  - (iii) TP-TP Primer set is difficult to design.
- (4) TP-FP primer set
  - (i) TP-FP primer set needs only three areas depending on the template sequence.
  - (ii) TP-FP Primer set needs a design of only one good TP because FP whose folded sequence can be designed in advance independently from template sequence **DOES NOT** control the reaction.
  - (iii) TP-FP Primer set is easy to design.

## Short time amplification and easy primer design(2)



# Office Action (1)

## • Summary of the Office Action

The examiner pointed out as follows;

- (1) TP are shown in Figure 4, step 1 and 2 (① and ② shown in below left) in Rabbani (EP0971039A2).
- (2) FP are shown in Figure 1, step 3 (③ shown in below right) in Rabbani.
- (3) Therefore, Claims 1 to 5 of the present invention lacks novelty (102(b)).

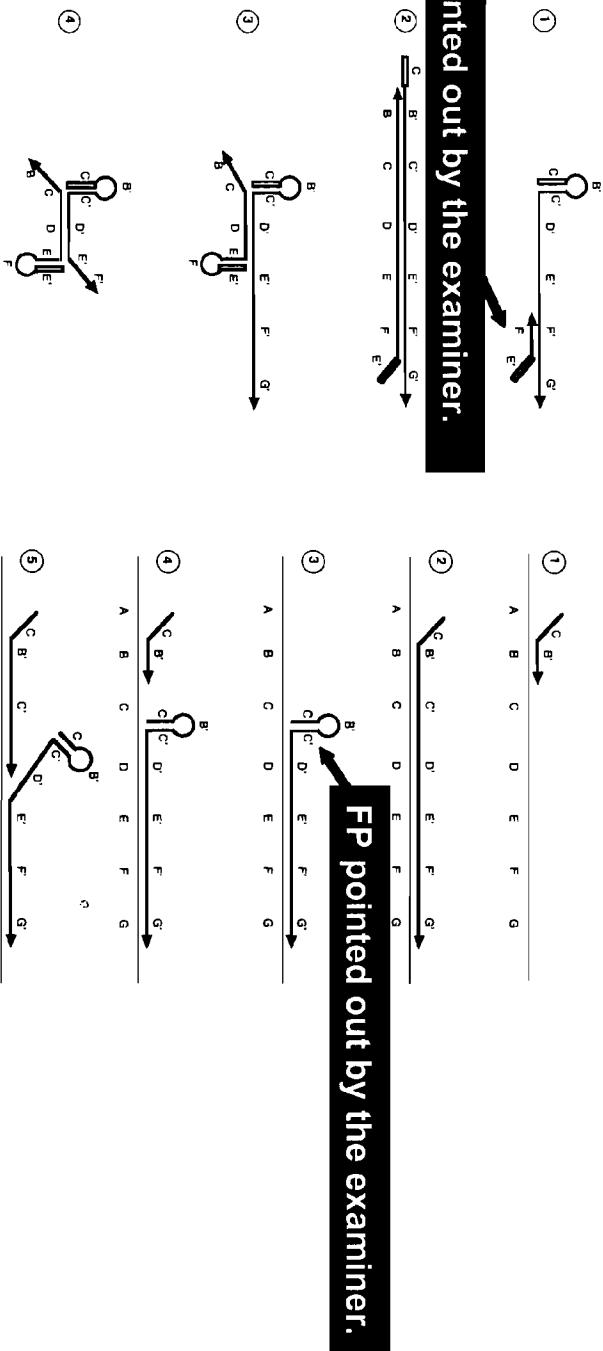


FIGURE 4

FIGURE 1

## Office Action (2); FIGURE 1 ③ in Rabbani is NOT FP.

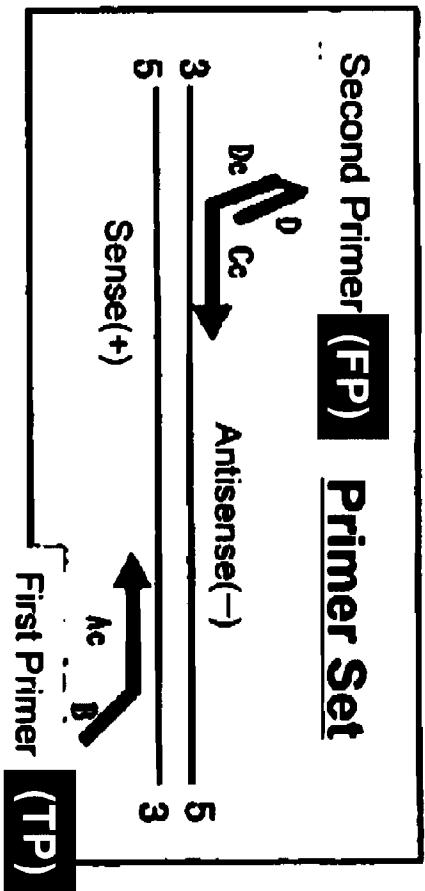
(1) FIGURE 1③ in Rabbani shows the elongation strand from TP.

strand from TP.

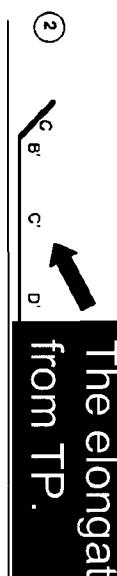
(2) Primer is different from

the elongation strand.

(3) Rabbani DOES NOT show the TP-FP primer set of the present invention.



\*Rabbani DOES  
NOT show FP.



from TP.

③

The elongation strand

from TP.

Stem-loop structure

of 5' portion in the

above elongation

strand from TP

(NOT FP).

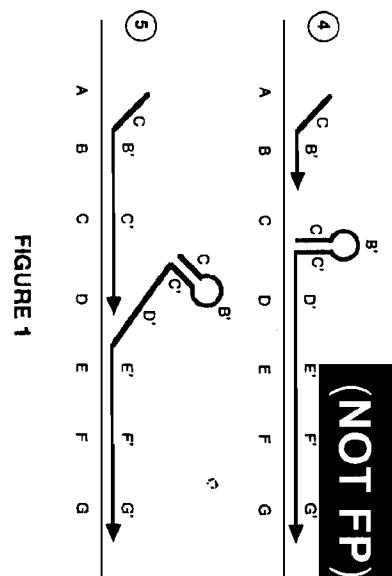
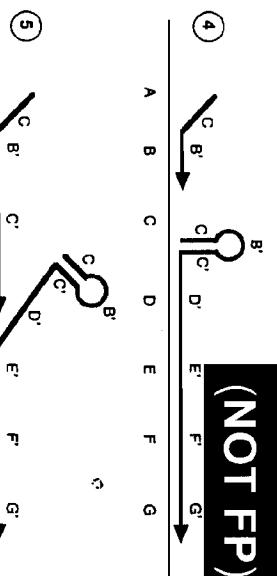


FIGURE 1

# In case FP is used in the FIGURE 1 in Rabbani.

